

# Supplementary Information

## Title

**Enhancement of immunomodulative effect of lactic acid bacteria on plasmacytoid dendritic cells with sucrose palmitate**

## Authors

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| Emulsifier                                | Hydrophilic group | Hydrophobic group      | Organic acid |
|-------------------------------------------|-------------------|------------------------|--------------|
| Emulsie P-100<br>(P-100)                  | monoglyceride     | stearate and palmitate | -            |
| Poem W-60<br>(W-60)                       | monoglyceride     | stearate               | tartrate     |
| Poem B-15V<br>(B-15V)                     | monoglyceride     | saturated fatty acid   | succinate    |
| Poem BS-20<br>(BS-20)                     | monoglyceride     | saturated fatty acid   | succinate    |
| Poem TRP-97RF<br>(TRP-97RF)               | triglyceride      | palmitate              | -            |
| RYOTO sugar ester P-1670<br>(P-1670)      | sucrose           | palmitate              | -            |
| RYOTO sugar ester S-1670<br>(S-1670)      | sucrose           | stearate               | -            |
| RYOTO sugar ester M-1695<br>(M-1695)      | sucrose           | myristate              | -            |
| RYOTO sugar ester O-1570<br>(O-1570)      | sucrose           | oleate                 | -            |
| RYOTO polyglycerol ester S-28D<br>(S-28D) | polyglyceride     | stearate               | -            |
| RYOTO polyglycerol ester M-7D<br>(M-7D)   | polyglyceride     | myristate              | -            |
| RYOTO polyglycerol ester L-100<br>(L-100) | polyglyceride     | laurate                | -            |

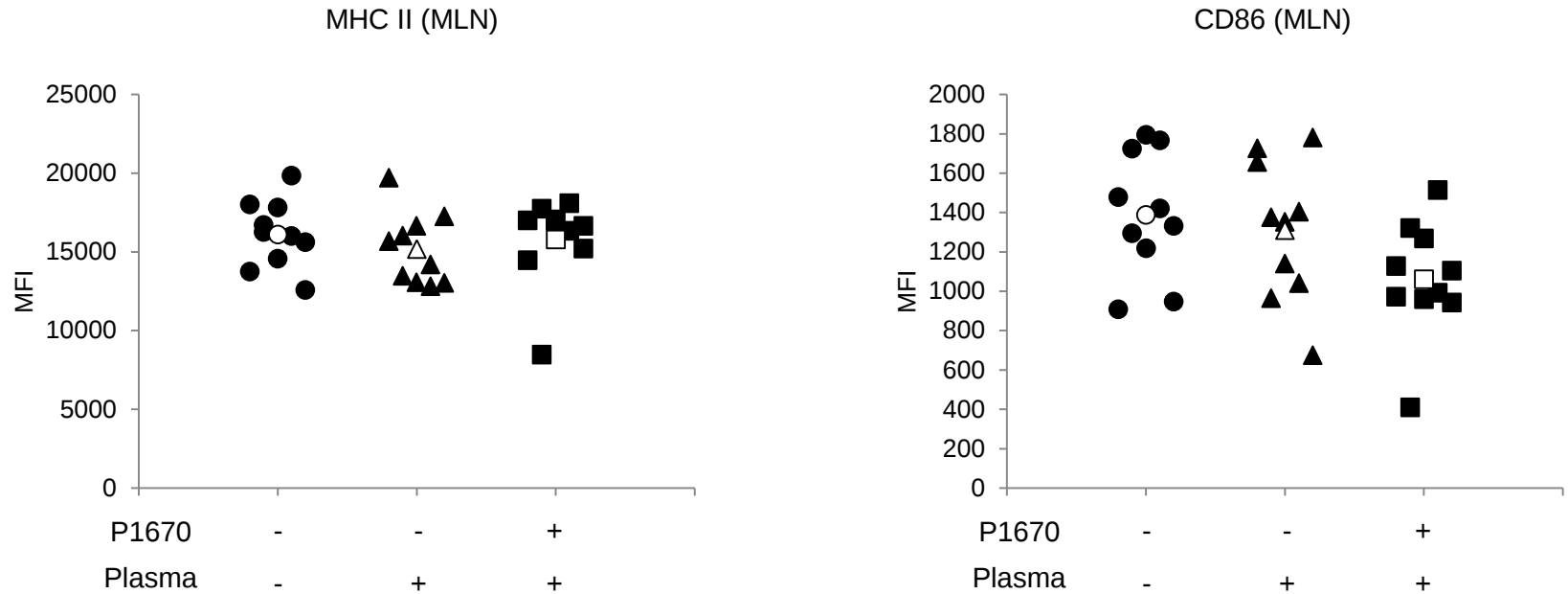
Supplementary Table 1 Emulsifiers used in this study.  
The major compounds contained in the tested emulsifiers are listed.

| No. | Plasma | Emulsifier | IFN- $\alpha$ (pg/mL) |       |      |    |
|-----|--------|------------|-----------------------|-------|------|----|
| 1   | -      | -          | 0.0                   | $\pm$ | 0.0  |    |
| 2   | +      | -          | 71.3                  | $\pm$ | 6.0  |    |
| 3   | +      | P1670      | 219.7                 | $\pm$ | 49.5 | ** |
| 4   | +      | S1670      | 151.5                 | $\pm$ | 17.9 | ** |
| 5   | +      | O1570      | 49.8                  | $\pm$ | 4.8  |    |

Supplementary Table 2 IFN- $\alpha$  production by BM-DCs stimulated with LC-Plasma mixed with emulsifiers. BM-DCs were cultured with 10  $\mu$ g/mL of LC-Plasma or LC-Plasma mixed with emulsifiers for 24 h. The concentration of IFN- $\alpha$  in the culture medium was measured by ELISA. The data show the mean  $\pm$  SD for triplicate wells. The statistical significance of differences among treatment groups was measured using one-way ANOVA with Dunnett's post-hoc test for comparison to the LC-Plasma alone group (\*\* $p < 0.01$ ).

| No. | Plasma | Emulsifier | IFN- $\alpha$ (pg/mL) |       |      |    |
|-----|--------|------------|-----------------------|-------|------|----|
| 1   | -      | -          | 0.0                   | $\pm$ | 0.0  |    |
| 2   | +      | -          | 104.4                 | $\pm$ | 10.1 |    |
| 3   | +      | P-100      | 128.5                 | $\pm$ | 11.7 |    |
| 4   | +      | W-60       | 144.1                 | $\pm$ | 9.1  | *  |
| 5   | +      | B-15V      | 156.3                 | $\pm$ | 21.5 | ** |
| 6   | +      | BS-20      | 147.1                 | $\pm$ | 10.1 | ** |

Supplementary Table 3 IFN- $\alpha$  production by BM-DCs stimulated with LC-Plasma mixed with emulsifiers. BM-DCs were cultured with 10  $\mu$ g/mL of LC-Plasma or LC-Plasma mixed with emulsifiers for 24 h. The concentration of IFN- $\alpha$  in the culture medium was measured by ELISA. The data show the mean  $\pm$  SD for triplicate wells. The statistical significance of differences among treatment groups was measured using one-way ANOVA with Dunnett's post-hoc test for comparison to the LC-Plasma alone group (\* $p$  < 0.05, \*\* $p$  < 0.01).



Supplementary Figure 1 Effects of P-1670 on mDCs activity in vivo. Mice were randomly divided into three groups consisting of ten mice each; control, LC-Plasma, and LC-Plasma mixed with P-1670. Each group was orally administered with the following treatments: distilled water for the control group, 1 mg of LC-Plasma for the LC-Plasma group, and 1 mg of LC-Plasma mixed with P1670 for the LC-Plasma mixed with P-1670 group. Twenty-four hours after the single administration, the mice were sacrificed and the cell surface expression of CD86 and MHC class II on the mDCs of mesenteric lymph nodes was measured by flow cytometry. The data show the mean  $\pm$  SD. The white-coloured marks show the average of each group.